(12) UK Patent Application (19) GB (11) 2 378 899 (13) A

(43) Date of A Publication 26.02.2003

(21) Application No 0120654.9

(22) Date of Filing 25.08.2001

(71) Applicant(s)
Thromb-X N.V.
(Incorporated in Belgium)
Leopold I straat 1 bus 21, B-3000 Leuven, Belgium

(72) Inventor(s)
Anne Angelillo-Scherrer
Peter Carmeliet
Desire Collen

(74) Agent and/or Address for Service
Thromb-X nv
c/o Desire Jose Collen,
Collingham Garden 28, LONDON,
SW5 0HN, United Kingdom

(51) INT CL[7]
A61K 38/36 // A61K 45/00 , A61P 7/06

(52) UK CL (Edition V)
A5B BHA B31X B31Y
U1S S2415

(56) Documents Cited
WO 1996/028548 A1 **US 5538861 A**
Proceedings of the National Academy of Sciences USA (2000), vol 97(22), pgs 12260-12265
Journal of Biological Chemistry (1996), vol 271(47), pgs 30022-30027

(58) Field of Search
UK CL (Edition T) **A5B**
INT CL[7] **A61K** 38/36 45/00, **A61P** 7/06, **C07K** 14/745
Other: **Online: CAS, BIOSIS, EMBASE, MEDLINE, SCISEARCH, EPODOC, WPI, PAJ, TXTUS0, TXTUS1, TXTUS2, TXTUS3, TXTEP1, TXTGB1, TXTWO1**

(54) Abstract Title
Treatment of anemia

(57) The present invention relates to a new method for the treatment of anemia based on the administration of the product of growth arrest-specific gene 6 (Gas6), its mutants, variants, active derivatives and the physiological tolerated salts of said Gas6 derivatives or of a group of biologically active substances inducing a GAS6 expression or releasing action or of a group of compounds that activate the Sky, Axl or Mer receptor tyrosine kinases. Furthermore the invention relates to a pharmaceutically effective composition to treat anemia. The composition comprises a pharmaceutically effective amount of a product of growth arrest-specific gene 6 (Gas6), or its mutant, variant, active derivative or the physiological tolerated salts of said Gas6 derivative or a biologically active substance that induces GAS6 expression or GAS6 release or a substance that activates the Sky, Axl or Mer receptor tyrosine kinases.

GB 2 378 899 A

TREATMENT OF ANEMIA

Field of the invention

The present invention relates to a new method for the treatment of anemia based on the administration of the product of growth arrest-specific gene 6 (Gas6), its mutants, variants, active derivatives and the physiological tolerated salts of these Gas6 derivatives or of a group of biologically active substances inducing a GAS6 expression or releasing action or of a group of compounds that activate the Sky, Axl or Mer receptor tyrosine kinases.

Background

The term *anemia* refers to a reduction below normal in hemoglobin level or red blood cells in the blood. Anemia is a major cause of morbidity and mortality. It may result from a reduced rate of production or increased rate of destruction of red blood cells or their loss from the circulation by bleeding. Although the cause of anemia can be a primary disorder of the production or survival of red blood cells, in many cases it is secondary to diseases of other systems.[1]

The mature red blood cell has two main functions. First, it must survive in the circulation for as long as possible, most of the time in vessels smaller than its own diameter. Second, it must both preserve its hemoglobin in state suitable for oxygen transport and adapt the amount of oxygen that is delivered to the needs of the tissues.[1]

The process by which erythroid cells are produced is called erythropoiesis. The production of mature red blood cells from pluripotent hematopoietic stem cells requires the coordinated action of different cytokine signaling pathway to assure controlled cell proliferation, survival, differen-

tiation and death. Under normal conditions, the whole process of erythropoiesis results in a red blood production rate such that the red cell mass in the body is kept constant. Erythropoiesis involves a great variety and number of cells at different stages of maturation, starting with the first stem cell progeny committed the erythroid differentiation and ending with the mature circulating red blood cell. Erythropoiesis occurs in distinct stages and anatomic sites during development. Primitive erythropoiesis begins around embryonic day 7 when red blood cells originate in the blood islands of the yolk sac. These cells express embryonic globins and are not dependent upon erythropoietin (EPO).[2,3] EPO is the major cytokine for definitive and adult erythroipoiesis. Definitive erythropoiesis starts at approximately day 10 when the site of red blood cell production shifts from the yolk sac to the fetal liver and is characterised by the production of non-nucleated erythrocytes expressing adult globin genes. At birth, erythroblastic islands in the bone marrow and the red pulp of the spleen become dominant sites of red blood cell production.

Gas6, the product of the growth arrest-specific gene 6 (*gas6*), is a new member of the vitamin K-dependent protein family.[4,5] Proteins belonging to this family are characterised by post-translational (-carboxylation of certain glutamic acid residues by a carboxylase, using vitamin K as cofactor. The (-carboxyglutamic acid (Gla)-containing module in prothrombin, coagulation factors VII, IX and X, protein C, protein S, protein Z and Gas6 allow these vitamin K-dependent proteins to bind to negatively charged phospholipid membranes.[6] Gas6 is structurally similar to protein S, but lacks a loop, crucial for the anticoagulant activity of protein S.[4] The latter is a co-factor for activated protein C, which inactivates the coagulation factors Va and VIIIa.[7] Genetic deficiency of protein S in man is one of the most severe inherited risk factors for thrombosis.[8]

Apart from a Gla-domain-dependent interaction with phospholipid membranes.[9] Gas6 also binds as a ligand to the receptor tyrosine kinases Axl (Ark, Ufo, Tyro7), Sky (Rse, Tyro3, Dtk, Etk,

Brt, Tif) and Mer (c-Mer, Eyk, Nyk)[10-14] by its carboxy-terminal globular G domains.[12] It has been implicated in reversible cell growth arrest,[4,5] survival,[15] proliferation,[15-17] and cell adhesion.[9,18,19] Mice with a triple deficiency of Axl, Sky and Mer are viable, but have not been reported to suffer spontaneous bleeding or thrombosis.[20]

We have recently shown that Gas6 deficient ($Gas6^{-/-}$) mice, generated by homologous recombination, are born at the expected mendelian frequency. $Gas6^{+/-}$ and $Gas6^{-/-}$ mice are viable, fertile, appear normal and show no obvious differences in size, weight or behaviour. No genotypic differences in litter size are observed [21]. These mice are protected against thrombosis because of a platelet dysfunction.[21]

Gas6 expression has been detected in hematopoietic tissue, both in hematopoietic (megakaryocytes and myelomonocytic precursors) and stromal (endothelial cells, fibroblasts, adipocytes) cells.[22] Gas6 receptor Axl is expressed in hematopoietic progenitors and bone marrow stromal cells, at low levels in monocytes, and in neoplastic cells of the myeloid lineage.[23] Axl and Sky are detectable at sites of embryonic hematopoiesis.[24] However, Gas6 is considered to have no mitogenic effect in the hematopoietic system, and not affecting the proliferative stimuli exerted by other hematopoietic growth factors.[22] More recently, monolayers of Gas6 expressing fibroblasts were shown to support the generation of colony-forming units in culture. This hematopoietic support is not vitamin K-dependent and soluble recombinant Gas6 does not substitute for coculturing the hematopoietic progenitors with Gas6 expressing fibroblasts.[25]

We have now found that Gas6 expression is required for the development of sufficient erythroid reserves in mice. $Gas6^{-/-}$ mice are not anemic but show a reduced level of blood reticulocytes. In addition, they have less cells of the erythroid lineage in adult bone marrow and spleen, and fetal liver at embryonic day E13.5. The decreased number of erythroid cells in the spleen has a slight

but significant impact on the spleen weight as $Gas6^{-/-}$ mice have a smaller spleen than their wild type counterpart. Immunohistochemical analysis of the spleen for Ter-119 (an erythrocytic lineage marker) shows a reduced size of the red pulp (site of red blood cell production) and a lower content in erythroid cells specifically labeled by anti-Ter-119 (an erythrocytic lineage marker) in $Gas6^{-/-}$ mice as compared to $Gas6^{+/+}$ mice. Even fetal liver isolated from $Gas6^{-/-}$ embryos is slightly smaller and contains less erythroid cells than fetal liver from $Gas6^{+/+}$ mice, $Gas6^{-/-}$, E13.5 embryos are not paler than $Gas6^{+/+}$ embryos, meaning that $Gas6^{-/-}$ embryos do not suffer from severe anemia. Erythroid progenitors are decreased in adult bone marrow and fetal liver at embryonic day E13.5 of $Gas6^{-/-}$ mice as compared to $Gas6^{+/+}$ mice. Finally, by inducing acute hemolytic anemia in mice, we demonstrate that erythropoietic response is markedly reduced in $Gas6^{-/-}$ mice.

Collectively, these data show that Gas6 can be used for the treatment of anemia. It constitutes a new class of promising antianemic drugs.

The present invention will be demonstrated in more detail in the following examples, which are however not intended to limit the scope of the invention.

Summary of the invention

One object of present invention is the use of an activator of the Sky, Axl or Mer receptor tyrosine kinases, for the manufacture of a medicament to treat a patient to prevent, reduce or cure anemia or the use of Gas6, or an analogue, mutant, variant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative for the manufacture of a medicament to treat a patient to prevent, reduce or cure anemia of said patient. Another object can also be the use of biologically active substances inducing a GAS6 expression or releasing action for the manufacture of a medicament

to treat a patient to prevent, reduce or cure anemia of said patient. This medicament can be supplemented with erythropoeitin. The anemia can be caused by or associated with various disorders such as chronic disease, chronic renal failure, aplastic anemia, hemolytic anemia, malignancies, endocrine deficiencies or can be caused by a specific treatment such as chemotherapy.

Another object of present invention is the use of Gas6, or an analogue, mutant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative, in combination with erythropoietin for the manufacture of an antianemic drug or an antianemic composition for autologous blood transfusion in a patient. These medicaments can be used to increase the survival rate of the patient suffering of anemia.

Another preferred embodiment of present invention is a method for the treatment, curing or prevention, wherein the patient to be treated receives an effective amount of Gas6, or an analogue, mutant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative, and for a suitable time to prevent, reduce or cure anemia or wherein the patient to be treated receives an effective amount biologically active substances inducing a GAS6 expression or releasing action and for a suitable time to prevent, reduce or cure anemia. This method may further comprise administering of erythropoietin.

Yet another preferred embodiment is an antianemic pharmaceutical composition comprising Gas6, or an analogue, mutant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative. The antianemic pharmaceutical composition can further comprise erythropoietin.

Description

EXAMPLE I: MICE DEFICIENT IN GAS6 (GAS6$^{-/-}$ MICE) HAVE A DECREASED RETICULOCYTE COUNT

Animal experiments were conducted according to the guiding principles of the American Physiological Society and the International Commitee on Thrombosis and Haemostasis[26]

Blood was collected under general anesthesia from retrobulbar plexus of wild type mice ($Gas6^{+/+}$ mice) or mice in which Gas6 expression was abolished by homologous recombination ($Gas6^{-/-}$ mice).[21] Reticulocyte counts were performed on smears of blood that had been stained with New Methylene Blue according to standard protocol (Sigma R4132). At least 1000 red blood cells were counted in each determination.

The data are represented as mean ± SEM of n determinations. The significance of differences was determined by unpaired Students'-test. Reticulocytes represented 24.6 ± 4.2 ‰ (n=8) of the circulating red blood cells in $Gas6^{+/+}$ mice and only 13.1 ± 3.1‰ (n=8) of the red blood cells in $Gas6^{-/-}$ mice ($p<0.001$). These data indicate that Gas6 is necessary for maintaining the reticulocyte count within the normal range.

EXAMPLE II: GAS6 DEFICIENT ($GAS6^{-/-}$) MICE HAVE REDUCED ERYTHROID RESERVES

Single-cell suspensions were obtained from bone marrow and spleen isolated from $Gas6^{+/+}$ and $Gas6^{-/-}$ mice. Ammonium chloride lysis of mature red blood cells was performed. Bone marrow and spleen cells (10^6) were incubated on ice with rat anti-mouse CD16/CD32 to block nonspecific binding to Fc receptors. Cell suspension was then stained with (PE)-conjugated-anti-Ter-119 antibody (PharMingen)for 30 min. at 4°C in 100 µl phosphate-buffered saline containing 0.2% BSA. Appropriate isotype control antibodies were used. Cell surface expression of Ter-119 was analyzed in a Becton Dickinson FACScan using CellQuest software.

Ter-119 is expressed by cells of the erythroid lineage, from proerythroblast to mature red blood cells. Ter-119 is expressed by 29 ± 3 % (mean ± SEM, n=3) of $Gas6^{+/+}$ bone marrow cells versus 17 ± 3 % (mean ± SEM, n=3) of $Gas6^{-/-}$ bone marrow cells ($p<0.05$). Similarly, Ter-119 is expressed by 6.0 ± 1.3 % (mean ± SEM, n=3) of $Gas6^{+/+}$ spleen cells versus 1.9 ± 0.5 % (mean ± SEM, n=3) of $Gas6^{-/-}$ spleen cells ($p<0.05$).

EXAMPLE III: THE NUMBER OF ERYTHROID PROGENITORS IS DECREASED IN THE BONE MARROW OF MICE DEFICIENT IN GAS6 ($GAS6^{-/-}$ MICE)

In vitro clonogenic assays for progenitor cells allows the study of distinct populations at different stages of development.

Single-cell suspensions were prepared from bone marrow or livers of day 13.5 embryos (E13.5) and counted in the presence of 3% acetic acid to lyse erythrocytes. Cell suspensions were mixed with MethoCult M3434 (StemCell Technologies, Vancouver) as described.[27] Cells were plated in 35 mm dishes and cultured at 37°C, 5% CO2. Colonies including burst-forming units erythroid (BFU-E, early erythroid progenitor) were scored at day 7. For the final progenitor cell colony-forming units erythroid (CFU-E) assay, cells were cultured in MethoCult 3230 containing 0.2 U/ml recombinant murine erythropoietin (R&D Systems) and colonies were scored at day 3.

In $Gas6^{+/+}$ mice the number of BFU-E per 10^5 bone marrow cells was 153 ± 44 (mean ± SEM, n=8), whereas the number of BFU-E per 10^5 bone marrow cells in $Gas6^{-/-}$ mice was 77 ± 35 (mean ± SEM, n=8). Thus, the number of colonies arising from bone marrow early progenitors (BFU-E) was reduced two-fold in $Gas6^{-/-}$ in comparison to $Gas6^{+/+}$ mice ($p<0.05$).

In addition, we studied $Gas6^{-/-}$ mice during fetal development, a time of rapid growth and little reserve capacity of the erythroid lineage. $Gas6^{+/+}$ E13.5 embryos contained 23 ± 7 $x10^3$ BFU-E per fetal liver (mean ± SEM, n=6). In contrast, the number of BFU-E per $Gas6^{-/-}$ fetal liver was 12 ± 3 $x10^3$ (mean ± SEM, n=6). Moreover,$Gas6^{-/-}$ embryos contained fewer final erythroid progenitor cells (colony-forming units erythroid, CFU-E colonies per fetal liver: 34 ± 8 $x10^3$ in $Gas6^{+/+}$ embryos, n=6 versus 17 ± 10 $x10^3$ in $Gas6^{-/-}$ embryos, n=6, $p<0.05$).

Thus, erythroid progenitors are decreased in adult bone marrow and fetal liver at embryonic day E13.5 of $Gas6^{-/-}$ mice as compared to $Gas6^{+/+}$ mice.

EXAMPLE IV: IMPAIRED RECOVERY AFTER ACUTE HEMOLYTIC ANEMIA OF GAS6 DEFICIENT MICE ($GAS6^{-/-}$ MICE)

Anemia was induced in $Gas6^{+/+}$ and $Gas6^{-/-}$ mice by intraperitoneal injection (0.5 or 2 mg /10 g body weight) of freshly prepared phenylhydrazine.[28] Phenylhydrazine hydrochloride (Sigma P6926) was dissolved in PBS at either 10 or 20 mg/ml and the pH was adjusted to pH 7.4 with NaOH. At day 3 following treatment with low dose of PHZ (two doses of 0.5 mg/10 g, 8 hours apart), $Gas6^{-/-}$ mice had a deeper depression in hematocrit (mean ± SEM: 29 ± 0.5 %, n=5, $p<0.001$) than $Gas6^{+/+}$ mice (hematocrit, mean ± SEM: 36 ± 2 %, n=5). In addition, their spleen (site of red cell production) was weighing less (mean ± SEM: 142 ± 27 mg n=4, $p<0.05$) as compared to $Gas6^{+/+}$ mice (mean ± SEM: 254 ± 28 mg, n=4), indicative of impaired erythropoiesis. Moreover, $Gas6^{-/-}$ mice were more susceptible to hemolysis induced by high dose (2 mg/10 g in one single dose) of PHZ as all $Gas6^{-/-}$ (n=10) succombed in comparison to 25 % $Gas6^{+/+}$ mice (n=10).

Taken together, these data indicate that the lack of Gas6 expression increase the susceptibility to acute hemolysis.

References to this application

1. Weatherall DJ, Provan AB. Red cells I: inherited anaemias. *Lancet*. 2000;355:1169-75.
2. Wu H, Liu X, Jaenisch R, Lodish HF. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. *Cell*. 1995;83:59-67.
3. Lin CS, Lim SK, D'Agati V, Costantini F. Differential effects of an erythropoietin receptor gene disruption on primitive and definitive erythropoiesis. *Genes Dev*. 1996;10:154-64.
4. Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *Mol Cell Biol*. 1993;13:4976-85.
5. Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. *Cell*. 1988;54:787-93.
6. Dahlback B. Blood coagulation. *Lancet*. 2000;355:1627-32.
7. Dahlback B. Protein S and C4b-binding protein: components involved in the regulation of the protein C anticoagulant system. *Thromb Haemost*. 1991;66:49-61.
8. Borgel D, Gandrille S, Aiach M. Protein S deficiency. *Thromb Haemost*. 1997;78:351-6.
9. Nakano T, Ishimoto Y, Kishino J, Umeda M, Inoue K, Nagata K, Ohashi K, Mizuno K, Arita H. Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6. *J Biol Chem*. 1997;272:29411-4.

10. Varnum BC, Young C, Elliott G, Garcia A, Bartley TD, Fridell YW, Hunt RW, Trail G, Clogston C, Toso RJ, et al. Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. *Nature*. 1995;373:623-6.

11. Godowski PJ, Mark MR, Chen J, Sadick MD, Raab H, Hammonds RG. Reevaluation of the roles of protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. *Cell*. 1995;82:355-8.

12. Nagata K, Ohashi K, Nakano T, Arita H, Zong C, Hanafusa H, Mizuno K. Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. *J Biol Chem*. 1996;271:30022-7.

13. Crosier KE, Crosier PS. New insights into the control of cell growth; the role of the AxI family. *Pathology*. 1997;29:131-5.

14. Chen J, Carey K, Godowski PJ. Identification of Gas6 as a ligand for Mer, a neural cell adhesion molecule related receptor tyrosine kinase implicated in cellular transformation. *Oncogene*. 1997;14:2033-9.

15. Goruppi S, Ruaro E, Schneider C. Gas6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts. *Oncogene*. 1996;12:471-80.

16. Li R, Chen J, Hammonds G, Phillips H, Armanini M, Wood P, Bunge R, Godowski PJ, Sliwkowski MX, Mather JP. Identification of Gas6 as a growth factor for human Schwann cells. *J Neurosci*. 1996;16:2012-9.

17. Nakano T, Higashino K, Kikuchi N, Kishino J, Nomura K, Fujita H, Ohara O, Arita H. Vascular smooth muscle cell-derived, Gla-containing growth-potentiating factor for Ca(2+)-mobilizing growth factors. *J Biol Chem*. 1995;270:5702-5.

18. Fridell YW, Villa J, Jr., Attar EC, Liu ET. GAS6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. *J Biol Chem.* 1998;273:7123-6.

19. McCloskey P, Fridell YW, Attar E, Villa J, Jin Y, Varnum B, Liu ET. GAS6 mediates adhesion of cells expressing the receptor tyrosine kinase Axl. *J Biol Chem.* 1997;272:23285-91.

20. Lu Q, Gore M, Zhang Q, Camenisch T, Boast S, Casagranda F, Lai C, Skinner MK, Klein R, Matsushima GK, Earp HS, Goff SP, Lemke G. Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature.* 1999;398:723-8.

21. Angelillo-Scherrer A, de Frutos P, Aparicio C, Melis E, Savi P, Lupu F, Arnout J, Dewerchin M, Hoylaerts M, Herbert J, Collen D, Dahlback B, Carmeliet P. Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. *Nat Med.* 2001;7:215-21.

22. Avanzi GC, Gallicchio M, Cavalloni G, Gammaitoni L, Leone F, Rosina A, Boldorini R, Monga G, Pegoraro L, Varnum B, Aglietta M. GAS6, the ligand of Axl and Rse receptors, is expressed in hematopoietic tissue but lacks mitogenic activity. *Exp Hematol.* 1997;25:1219-26.

23. Neubauer A, Fiebeler A, Graham DK, O'Bryan JP, Schmidt CA, Barckow P, Serke S, Siegert W, Snodgrass HR, Huhn D, et al. Expression of axl, a transforming receptor tyrosine kinase, in normal and malignant hematopoiesis. *Blood.* 1994;84:1931-41.

24. Crosier PS, Freeman SA, Orlic D, Bodine DM, Crosier KE. The Dtk receptor tyrosine kinase, which binds protein S, is expressed during hematopoiesis. *Exp Hematol.* 1996;24:318-23.

25. Dormady SP, Zhang XM, Basch RS. Hematopoietic progenitor cells grow on 3T3 fibroblast monolayers that overexpress growth arrest-specific gene-6 (GAS6). *Proc Natl Acad Sci U S A*. 2000;97:12260-5.

26. Giles AR. Guidelines for the use of animals in biomedical research. *Thromb Haemost*. 1987;58:1078-84.

27. Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell*. 1998;93:397-409.

28. Lim SK, Kim H, bin Ali A, Lim YK, Wang Y, Chong SM, Costantini F, Baumman H. Increased susceptibility in Hp knockout mice during acute hemolysis. *Blood*. 1998;92:1870-7.

CLAIMS

What we claim is:

1. The use of an activator of the Sky, Axl or Mer receptor tyrosine kinases, for the manufacture of a medicament to treat a patient to prevent, reduce or cure anemia.
2. The use of Gas6, or an analogue, mutant, variant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative for the manufacture of a medicament to treat a patient to prevent, reduce or cure anemia of said patient.
3. The use of biologically active substances inducing a GAS6 expression or releasing action for the manufacture of a medicament to treat a patient to prevent reduce or cure anemia of said patient.
4. The medicament of the claims 1,2,3 or 4 comprising erythropoietin.
5. The use according to any of the claims 1 to 4, whereby the anemia results from a condition of chronic disease.
6. The use according to any of the claims 1 to 4, whereby the anemia results from a condition of chronic renal failure.
7. The use according to any of the claims 1 to 4, whereby the anemia is an aplastic anemia.
8. The use according to any of the claims 1 to 4, whereby the anemia is a hemolytic anemia.
9. The use according to any of the claims 1 to 4, whereby the anemia is associated with malignancies.
10. The use according to any of the claims 1 to 4, whereby the anemia is associated with endocrine deficiencies.

11. The use according to any of the claims 1 to 4, whereby the anemia is caused by chemotherapy.

12. The use of Gas6, or an analogue, mutant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative, in combination with erythropoietin for the manufacture of an antianemic drug or an antianemic composition for autologous blood transfusion in a patient.

13. The use according to any of the claims 1 to 12, to increase the survival rate of the patient suffering of anemia.

14. The use according to any one of the preceding claims, wherein the patient to be treated is a mammal, preferable a human being.

15. A method for the treatment, curing or prevention anemia according to each of the previous use claims, wherein the patient to be treated receives an effective amount of Gas6, or an analogue, mutant or derivative thereof, or a physiological tolerated salt of said Gas6 derivative, and for a suitable time to prevent, reduce or cure anemia.

16. A method for the treatment, curing or prevention anemia according to each of the previous use claims, wherein the patient to be treated receives an effective amount biologically active substances inducing a GAS6 expression or releasing action and for a suitable time to prevent, reduce or cure anemia.

17. The method of claim 15 or 16, further comprising administering of erythropoietin.

18. An antianemic pharmaceutical composition comprising Gas6, or an analogue, mutant or derivative thereof or a physiological tolerated salt of said Gas6 derivative.

19. The antianemic pharmaceutical composition of claim 16 further comprising erythropoietin.

INVESTOR IN PEOPLE

Application No: GB 0120654.9
Claims searched: 1-19

Examiner: Richard Sewards
Date of search: 26 February 2002

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T): A5B

Int Cl (Ed.7): A61K 38/36, 45/00, A61P 7/06, C07K 14/745

Other: Online: CAS, BIOSIS, EMBASE, MEDLINE, SCISEARCH, EPODOC, WPI, PAJ, TXTUS0, TXTUS1, TXTUS2, TXTUS3, TXTEP1, TXTGB1, TXTWO1

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	WO 96/28548 A1 (GENENTECH) see pg 3 ll 18-23, pg 34 ll 4-13, pg 35 ll 13-27, Examples 7, 11 & 12	1-19
A	US 5538861 (AMGEN) see col 2 l 50 - col 3 l 6	
X	Proceedings of the National Academy of Sciences USA (2000), vol 97(22), pgs 12260-12265; Dormady et al; see whole document	1-19
A	Journal of Biological Chemistry (1996), vol 271(47), pgs 30022-30027; Nagata et al; see abstract and Discussion	

X Document indicating lack of novelty or inventive step
Y Document indicating lack of inventive step if combined with one or more other documents of same category.
& Member of the same patent family
A Document indicating technological background and/or state of the art.
P Document published on or after the declared priority date but before the filing date of this invention.
E Patent document published on or after, but with priority date earlier than, the filing date of this application.